Supporting Text

The constant production hypothesis provides signals for cells to begin differentiation from ground to vascular according to a mathematical model. To develop the reader's intuition, we now develop several properties of this model in one dimension. We begin with convergence properties at equilibrium, to demonstrate plausibility of the parameters in *Result 1* and then illustrate the stability of connections forming according to the dynamics implied by *Schema 1.* The numerical values were chosen for computational convenience; although they highlight robustness over a range of parameter values, we emphasize that reliable estimates of concentration, gradient, and diffusion values are currently unknown.

1 Behavior of the model in 1D

Suppose $n+1$ cells are arranged in a file (path graph) indexed 0 through n from left to right. Suppose each cell except the 0^{th} produces a substance s at a rate K which is fixed. Denote by $c(i,n)$ the concentration in cell i and let $c(0,n) = 0$ at all times. The substance is allowed to diffuse from cell to cell if they are connected and the associated coefficient is D. Let $\alpha = \frac{K}{D}$ and $\Delta c(i,n) = c(i-1,n) - c(i,n)$. The dynamics are thus given by (dropping the n)

$$c_t(i) = D(c(i-1) - 2c(i) + c(i+1)) + K.$$

Proposition 1. *At steady-state, i.e., $c_t = 0$, $c(0) = 0$ imposes the following:*

$$\begin{aligned} c(i) &= \alpha\left(i(n+\frac{1}{2}) - \frac{1}{2}i^2\right) && (1)\\ \Delta c(i) &= \alpha(n-i) && (2)\\ c(1) = \Delta c(1) &= \alpha n && (3)\\ c(n) &= \alpha\frac{n(n+1)}{2}, && (4) \end{aligned}$$

where $\alpha = K/D$.

Proof. Rearrange the terms in the dynamics to obtain:

$$c_t = D((c(i-1) - c(i)) + (c(i+1) - c(i))) + K.$$

So, $c_t = 0$ implies

$$\begin{aligned} c(i-1) - c(i) &= c(i) - c(i+1) - \tfrac{K}{D} \\ \Longrightarrow \qquad \Delta c(i) &= \Delta c(i+1) + \alpha, \end{aligned}$$

and at the end of the chain $\Delta c(n) = \alpha$ because there is no right connection. Thus, $\Delta c(n-j) = j\alpha$. Since $c(1) = \Delta c(1)$ (because $c(0) = 0$), we have

$$\begin{aligned} c(i) &= \alpha \textstyle\sum_{j=1}^{i-1} \Delta c(j) \\ &= \alpha \textstyle\sum_{j=0}^{i-1} (n-j) \\ &= \alpha \left(i(n+\tfrac{1}{2}) - \tfrac{1}{2} i^2 \right), \end{aligned}$$

and the rest follows. □

Proposition 2. *The claims in Proposition 1 are true for a file of cells with $2n$ cells where $c(2n+1) = 0$ is also imposed.*

Further analysis of the 2D case may be found in ref. 1.

2 Illustration of Vein Formation Dynamics

The constant production hypothesis provides a framework for illustrating the vein formation dynamics. Movies 1–5 are simulations with a single parameter changed. The demonstration is of the behavior of c over a file of cells. Each cell produces the substance at the same constant rate $K = 0.001$ equally inside it. Diffusion within a cell and through interfaces is accounted for by discretizing the interior and treating it as four compartments. The diffusion coefficient between interior compartments is $D_{interior}$, and $D_{interface}$ is the one between two cells (compartments of adjacent cells). The simulation is initiated at $c = 0$ for all compartments of all cells; and the two ends of the string of cells are held at a constant value: $c_R = 0$ for the rightmost end, and $c_L = 0.01$ for the other one. The dynamics of the distribution of c over the compartments and cells is simulated using a time step of $\Delta t = 0.01$ and the following update rule:

$$c(i) = c(i) + \Delta t[D_{i|i-1}(c(i-1) - c(i)) + D_{i|i+1}(c(i+1) - c(i))],$$

where $D_{i|j}$ denotes the diffusion coefficient between compartment i and compartment j. The blue curve in Fig. 6 shows the distribution of c approximately at convergence. The step-like shape is due to making

$D_{interior} = 800 D_{interface}$, with $D_{interior} = 0.04$, and allows one to clearly see where each cell starts and the next begins.

The movies show how applying *Schema 1* in the work can result in vascular strands that connect for a wide range of parameter values. The simulations start as equilibrium is approached. The threshold for differentiation is chosen to be the difference in concentration Δc at the right end; this is where Δc is largest on the blue curve. As soon as the threshold is exceeded, $D_{interface}$ is changed to $D^{new}_{interface} = M D_{interface}$ and the simulation proceeds as described.

Each of the five movies corresponds to a different value of M. When a change of $D_{interface}$ occurs, the movie shows concentration decrease much faster within that cell than the rest. This demonstrates graphically the differentiation "front." Connection robustness is observed for $M \geq 30$; simulations with $M = 30$ (Movie 3), $M = 40$ (Movie 4) and $M = 60$ (Movie 5) demonstrate connections. However, observe that for $M = 10$ (Movie 1) and $M = 20$ (Movie 2) the strand starting from the right never connects to the strand starting from the left: there is never a sharp decay of the last cell due to an increase of $D_{interface}$ on either side of the cell. Our model thus predicts an intimate relationship between timing, shape, size and vascular connectivity.

A complete analysis in 2D of this phenomenon is beyond the scope of this paper. We simply note that, just as these simulations illustrate varied dynamics with parameter changes, in 2D different parameter values combine to result in more or less regular subdivisions of areoles. In real leaves, the regularity of subdivision was used by Hickey to propose "leaf ranks" as an alternate character for classification (see [2] for references and descriptions).

3 Whole-leaf Behavior

In the main text we showed that it is possible to obtain predictions of new vascular strands using Schema 1 and a single threshold τ. However, to achieve this we required that K and D be slightly different from areole to areole: $K \mapsto \frac{1}{\sqrt{\mu}} K$ and $D \mapsto \sqrt{\mu} D$ (Fig. 5). As *Result 1* of the main text suggests, similar results should be obtained if we either (1) only vary the production rate by $K \mapsto \frac{1}{\mu} K$ or (2) only vary the diffusion coefficients $D \mapsto \mu D$. In particular, any one of these schemes will achieve $\frac{K}{D} \mapsto \frac{1}{\mu} \frac{K}{D}$. In Fig. 7 we demonstrate the effect of the two schemes by taking μ from Fig. 5 for each of the two stages shown there. Compare Fig. 7*B* with Fig. 7*D* with Fig. 5*C*, and Fig. 7*G* with Fig. 7*I* with Fig. 5*E*. We observe that there are only minor differences. In the absence of reliable parameter estimates these results point

to a falsifiable parameter relationship, which might be of interest to the experimental community.

4 Simulation of c in a young leaf

Cells were assumed to be square of unit volume, and their interfaces were the eight neighbors (lateral and diagonal). In Figs. 1 and 5 vascular interfaces have diffusivity $D_{vasc} = 5000 D_{ground}$, and in Fig. 8 $D_{vasc} = 100 D_{ground}$. Interfaces between two vascular cells use D_{vasc} and all other interfaces use $D_{ground} = 1(area)(time)^{-1}$. The c-vascular cells in Figs. 3 and 4 were assumed to be sinks. Each interface is assumed to be of unit area and each cell produces substance s at a rate of $K = 1(mass)(time{*}volume)^{-1}$. We define the matrix M as M_{ij}, the diffusivity between point i and point j for $i \neq j$, and $M_{ii} = -\sum_{j \in cells, j \neq i} M_{ij}$. If cell i is a sink, then $M_{ii} = 1$ and $\forall j \neq i,\ M_{ij} = 0$. The steady-state solution of Eq. **1** of the main text can then be obtained by solving for c in the linear system $Mc = -\vec{K}$, where $\vec{K}$ is the vector containing the production of each cell i. Similarly, iterating $c^{next} = c^{current} + dt(Mc^{current} + \vec{K})$ approximates the temporal behavior of c. The simulation in Fig. 9 involves adding an amount to one cell in $c^{current}$ and then iterating.

The predictions of new c-vascular strand creation are obtained in two ways: direct application of *Schema 1* or by drawing integral curves. In Fig. 3, the result of the first approach is shown. The concentration is updated iteratively as described above, and, after each step, all interfaces with $\Delta c > \tau$ are updated (converting the diffusion constant from D_{ground} to D_{vasc}). In the second approach, the gradient vector field of c is used to draw the integral curves initialized at a point on the boundary where $\|\nabla c\|$ is locally maximal (see Fig. 4 *A* and *E* for examples). Given a solution c as above, the vector field $\mathbf{F} = (\frac{\partial c}{\partial x}, \frac{\partial c}{\partial y})$ is approximated on a square lattice with spacing h. The curves are obtained by the iteration $P^{(t+1)} = P^{(t)} + \Delta t \frac{\mathbf{F}(P^{(t)})}{\|\mathbf{F}(P^{(t)})\|}$ where $P^{(0)}$ is the starting point and $\Delta t = 0.3h$ is the time step. At nonlattice points $\mathbf{F}$ is interpolated. This approximates the program described in *Schema 1* since the interface to first exceed a threshold while cell P is being drained will be the one in the direction of $\mathbf{F}(P)$. Notice that $P^{(t+1)}$ will reach a local maximum of c (where $\nabla c = 0$) and never leave it (see Fig. 4 *B* and *F*). This is the natural stopping point in all simulations.

References

[1] Dimitrov, P. & Zucker, S. W. (2006) *On a Differential Equation Arising in Plant Vascular Biology* (Yale Univ., New Haven, CT) Yale Computer Sci. Tech. Rep. 1345.

[2] Leaf Working Group (1999) *Manual of Leaf Architecture – Morphological Description and Categorization of Dycotiledonous Angiosperms.* Smithsonian Institute, Washington, DC.